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Diversity of Plant Pathogens and Soil Microbes Associated with *Emblica officinalis* in Nokrek Biosphere Reserve of Meghalaya

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Abstract The fruit plant *Emblica officinalis* Gaertn. commonly known as wild gooseberry is one of the common plants growing in Nokrek Biosphere Reserve, a biodiversity hotspot, located in the Garo Hills of Meghalaya, India. It is a hardy plant with high nutritive and therapeutic value. Fruits are a rich source of vitamin C and highly valued for indigenous medicine. The objective of the study was to identify pathogenic microbes infecting this fruit plant and soil microbes inhabiting the rhizosphere at different soil depths ; and the relationship between soil microbes and occurrence of plant pathogens. Eight diseases caused by fungal pathogens were observed in plants of *Emblica officinalis* in the three zones of Nokrek Biosphere Reserve during pre-monsoon and post-monsoon season. Soil microbes were more in number in the topsoil (0—15 cm) of the rhizosphere of *Emblica officinalis* compared to subsoil (15—30 cm) in all three zones of the Biosphere Reserve. Soil microbes like *Acremonium falciforme*, *Aspergillus niger*, *Trichothecium roseum* and *Aspergillus versiclor* exhibited positive influence on the occurrence of certain plain pathogens like *Sphaerothicamors-uvae*, *Fusarium oxysporum*, *Ravenelia emblicae, Botrytis cineria* and *Penicillium islandicum*. However, the same soil microbes showed an antagonistic effect on plant pathogens *Phomaputanium* and *Alternaria alternata*. Among the identified soil microbes *Aspergillus flavus*, *Penicillium digitatum* and *Rhizopus nigricans* were the dominant ones.

Keywords Nokrek Biosphere Reserve, *Emblica officinalis*, Plant pathogens, Soil microbes.

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Introduction

Emblica officinalis Gaertn. commonly known as wild gooseberry is a hardy plant popular for its nutritive and therapeutic value. It is a deciduous, small or medium size tree with pale yellow, globose and fleshy fruits with a hard seed inside. Fruits range from 1.4–3.0 cm in diameter and are a rich source of vitamin C. The bark, leaves and fruits are used in dyeing cotton fabrics and in tanning. Dried fruits are considered useful in treating diarrhoea, dysentery and hemor-

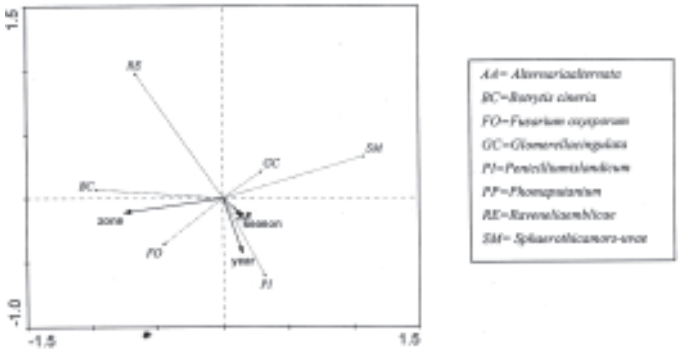

Fig. 1. Effect of zone, season and year on occurrence of plant pathogens in *Emblica officinalis*.

rhage. The seeds are used in treatment of various respiratory disorders like asthma and bronchitis [1]. The fruits are anabolic, antioxidative, expectorant and antiemetic. In folk medicine, the fruits are used for curing mouth ulcer, eye disease, acidity, indigestion, constipation, piles, baldness, cough and cold, headache, diabetes, hysteria, and for purifying blood. *Emblica officinalis* Gaertn. is one of the common plants growing in Nokrek Biosphere Reserve. The objective of the study was to find out the common disease causing pathogens and the composition of soil microbes in the rhizosphere of this plant as well as to assess the relationship between soil microbes and occurrence of plant pathogens.

Materials and Methods

The study was conducted in Nokrek Biosphere Reserve situated in between 25°25´–25°30´N latitudes and 90°15´–90°35´E longitudes at an elevation of about 4650 feet above sea level and stretched over an area of 820 sq km, located in the Garo Hills of Meghalaya, India. The Biosphere Reserve is divided into three zones : the core zone which is known as Nokrek National Park with an area of 47.48 sq km, the buffer zone covering an area of 227.92 sq km followed by transition zone with an area of 544.60 sq km. March—May are the hottest months and December to February are the coolest months. Both south-west and north-eastern monsoons bring rain to this area. The temperature varied from 8°C–38°C in the year 2014 and 10°C–36°C in 2015. The total rainfall in 2014 was 2778.9 mm and 3809.9 mm in 2015.

Five plants of *Emblica officinalis* were selected randomly from each zone of the Biosphere Reserve. Diseased plant parts were collected and pathogens were identified during pre-monsoon and post monsoon period of years 2014 and 2015. The diseased plant parts were cultured in the laboratory in different media under aseptic conditions. For isolation of fungal pathogen from diseased plant samples, single spore technique and single hyphal tip technique of culturing were used. The isolated fungal pathogen was then purified by streaking and single spore isolation method. Pathogenicity tests were conducted thereafter by pin prick method [2]. Pathogenicity was confirmed by Koch's postulates.

Soil samples were also collected during the same period from two different depths of the rhizosphere viz. 0—15 cm and 15—30 cm, from four directions of the plant with the help of soil auger. The soil samples collected from four directions of each depth were combined to form a composite sample. A representative sample was drawn from the composite sample for culturing and identification of microbes. Soil microorganisms were identified by the Direct method and Plate method. Isolation from soil samples were carried out by dilution plate method [3] using appropriate media. The colonies were identified by studying the macro and micro morphological characteristics.

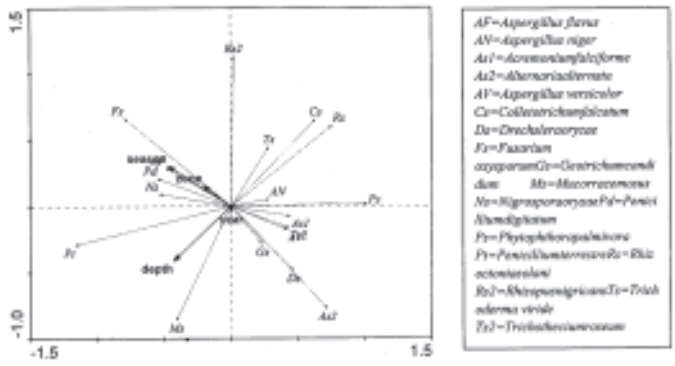

Fig. 2. Effect of zone, soil depth, seasons and year on occurrence of soil microbes in the rhizosphere of *Emblica officinalis*.

Microphotographs of the slides from the colony were taken and compared with standard literature for proper identification of microbes.

Statistical analysis

Redundancy Analysis (RDA) was followed to study the occurrence of plant microbes and soil microbes. Canonical Correspondence Analysis (CCA) was used to find out the relationship between soil microbes and occurrence of plant pathogens [4].

Results and Discussion

Observations during pre-monsoon period of years 2014 and 2015 revealed that the highest disease incidence occurred in the core zone followed by transition zone and buffer zone. However, during post monsoon season highest disease incidence was recorded in transition zone followed by core zone and buffer zone. Eight diseases caused by fungal pathogens were identified in the three zones of Nokrek Biosphere Reserve in both the seasons. Zone had a significant influence on occurrence of plant pathogens in *Emblica officinalis* while season and year did not have any significant effect. With the change in zone from core to buffer and transition, plant microbes like *Fusarium oxysporum*, *Botrytis cineria* and *Ravenelia emblicae* showed significant increase in their occurrence while *Glomerella cingulata*, *Sphaerothicamors-uvae*, *Phomaputanium* and *Alternaria alternata* showed a negative trend (Fig. 1).

Soil microbes were less at 15—30 cm depth (subsoil) in all three zones of the Biosphere Reserve compared to 0—15 cm depth (topsoil). Among the soil microbes identified in the three zones, *Aspergillus flavus*, *Penicillium digitatum* and *Rhizopus nigricans* were the dominant ones. Soil depth and season had a significant effect on occurrence of soil microbes in the rhizosphere of *Emblica officinalis*. Figure 2 shows that with an increase in soil depth, the probability of occurrence of *Mucorracemosus*, *Penicillium terrestre*, *Geotrichum candidum*, *Drechslera oryzae*, *Alternaria alternata*, *Aspergillus versicolor*, *Trichothecium roseum* and *Acremonium falciforme* increased significantly. The occurrence of microbes like *Phytophthora palmivora*, *Aspergillus niger*, *Rhizoctonia solani*, *Colletotrichum falcatum*, *Trichoderma viride*, *Rhizopus nigricans*, *Fusarium oxysporum*, *Aspergillus flavus*, *Penicillium digitatum* and *Nigrospora oryzae* was observed to be more in top soil (0—15 cm depth). Change in season from pre-monsoon to post monsoon had a positive effect on occurrence of soil microbes like *Aspergillus flavus*, *Fusarium oxysporum*, *Penicillium digitatum*, *Nigrospora oryzae*, *Penicillium terrestre* and *Mucorracemosus* while other microbes exhibited negative trend i.e. their occurrence was more in pre-monsoon period. Other independent factors like zone and year of ob-

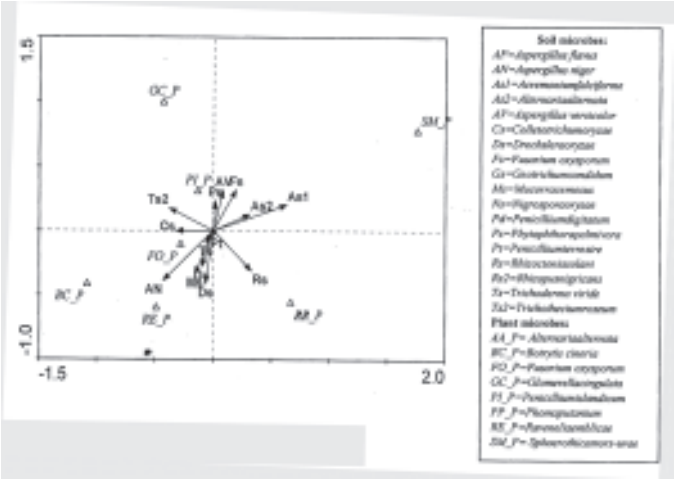

Fig. 3. Association of soil microbes at 0—15 cm soil depth of rhizosphere with plant pathogens of *Emblica officinalis*.

servation did not haveany significant effect on occurrence of soil microbes.

A significant association was observed between soil microbes viz., *Acremonium falciforme*, *Aspergillus* niger and *Trichothecium roseum* at 0—15 cm soil depth with some plant pathogens. Figure 3 clearly shows the strong association of soil microbe *Acremonium falciforme* with plant pathogen *Sphaerothicamors-uvae* ; soil microbe *Aspergillus niger* with plant pathogens *Fusarium oxysporum*, *Ravenelia emblicae* and *Botrytis cineria* and soil microbe *Trichothecium roseum* with plant pathogen *Penicillium islandicum*. However, plant pathogens *Phomaputanium* and *Alternaria alternata* showed negative probability of occurrence in the presence of soil microbes *Acremonium falciforme*, *Aspergillus niger* and *Trichothecium roseum*. As soil depth increased to 15—30 cm, soil microbe *Aspergillus niger* showed significant positive influence on occurrence of plant pathogen *Sphaerothicamors-uvae* while soil microbe *Aspergillus versiclor* exhibited a positive association with plant pathogen *Penicillium islandicum* (Fig. 4).

The plant pathogens infecting *Emblica officinalis* in all the three zones of Nokrek Biosphere Reserve during pre-monsoon and post monsoon periods of years 2014 and 2015 were fungal pathogens. Out of the three independent factors viz., zone, season and year, zone exhibited significant effect on the occurrence of plant pathogens. The importance of topographical factors was emphasized in the development of a fungal pathogen *Mycenacitricolor* causing American leaf spot of coffee, which favored regions between 1,100 and 1,500 m above sea level [5].

Observations revealed that soil depth had significant effect on the distribution of soil microbes. Soil microbes in the rhizosphere of *Emblica officinalis* were found to be significantly more in number in the topsoil (0—15 cm depth) compared to subsoil (15—30 cm depth) in all three zones of the Biosphere Reserve in pre-monsoon and post monsoon seasons during both the years of observation. Seasonal and depthwise variation in bacterial and fungal populations were studied in three arecanut based traditional agroforestry systems practised by two ethnic communities and reported that microbial counts were higher in top soil [6]. Seasonal and depthwise variations in soil bacterial and fungal populations were also studied in Dibru-Saikhowa Biosphere Reserve Forest of Assam where greater bacterial and fungal counts were observed in the surface layer of soil [7]. Generally topsoil contains high organic matter which in the presence of adequate moisture supply is acted upon by the microorganisms to decompose the com-

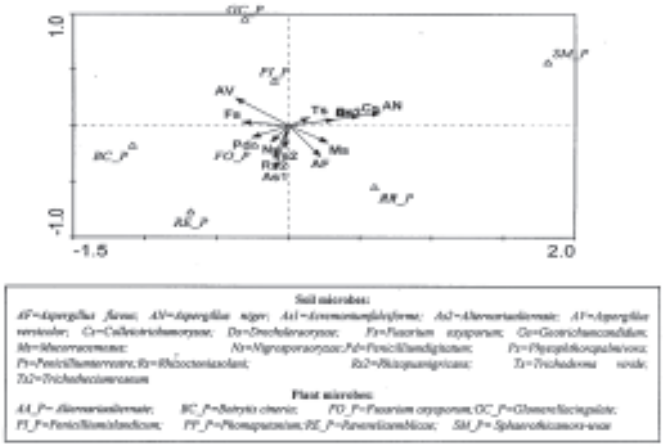

Fig. 4. Association of soil microbes at 15—30 cm soil depth of rhizosphere with plant pathogens of *Emblica officinalis*.

plex organic residues into simpler forms ; hence, microbial counts are generally higher in the surface soil layer as compared to lower depths [8].

It was observed that season had a significant effect on the occurrence of soil microbes in the rhizosphere of *Emblica officinalis*. Microbial abundance in the rhizosphere of different plant species varied greatly across season [9]. Soil microbial community structure and function are known to be sensitive to changes in both temperature and water availability and are therefore predicted to be responsive to such alterations of climatic system [10]. Among the soil microbes identified in the three zones, *Aspergillus flavus*, *Penicillium digitatum* and *Rhizopus nigricans* were the dominant ones. Dominance of genus *Aspergillus*, *Penicillium* and *Rhizopus* was also reported by other scientists in their study sites [6, 7].

A close relationship was observed between soil microbes inhabiting the rhizosphere of *Emblica officinalis* and pathogens infecting these plants in the Biosphere Reserve. Soil microbes like *Acremonium falciforme*, *Aspergillus niger*, *Trichothecium roseum* and *Aspergillus versiclor* exhibited positive influence on the occurrence of certain plant pathogens like *Sphaerothicamors-uvae*, *Fusarium oxysporum*, *Ravenelia emblicae Botrytis cineria* and *Penicillium islandicum*. However, the same soil microbes showed an antagonistic effect on plant pathogens *Phomaputanium* and *Alternaria alternata.*

Conclusion

Eight diseases caused by fungal pathogens were identified in *Emblica officinalis* plants in the three zones of Nokrek Biosphere Reserve in pre-monsoon and post-monsoon seasons. Zone played a significant role on occurrence of plant pathogens. Soil depth and season had significant effect on occurrence of soil microbes in the rhizosphere of *Emblica officinalis*. Soil microbes in the rhizosphere of *Emblica officinalis* were more in topsoil (0—15 cm) than the subsoil (15—30 cm) in core, buffer and transition zone of the Biosphere Reserve in pre-monsoon and post-monsoon seasons. Among the identified soil microbes, *Aspergillus flavus*, *Penicillium digitatum* and *Rhizopus nigricans* were found in all three zones. Soil microbes like *Acremonium falciforme*, *Aspergillus niger*, *Trichothecium roseum* and *Aspergillus versiclor* exhibited positive influence on the occurrence of certain plant pathogens like *Sphaerothicamors-uvae*, *Fusarium oxysporum*, *Ravenelia emblicae Botrytis cineria* and *Penicillium islandicum*. However, the same soil microbes showed an antagonistic effect on plant pathogens *Phomaputanium* and *Alternaria alternata*.

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